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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/065,610	11/04/2002	Chi-Ming Chen	9668-US-PA	2460

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JIANQ CHYUN INTELLECTUAL PROPERTY OFFICE
7 FLOOR-1, NO. 100
ROOSEVELT ROAD, SECTION 2
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EXAMINER

WOOLWINE, SAMUEL C

ART UNIT PAPER NUMBER

1637

DATE MAILED: 09/13/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

10/065,610

Applicant(s)

CHEN, CHI-MING

Examiner

Samuel Woolwine

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.138(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
 - If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
 - If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
 - Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 03 March 2003.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-15 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-15 is/are rejected.
- 7) ☒ Claim(s) 15 is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 04 November 2002 is/are: a) ☐ accepted or b) ☒ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All b) ☐ Some * c) ☐ None of:
1. ☒ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date _____.
- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____.
- 5) ☐ Notice of Informal Patent Application (PTO-152)
- 6) ☐ Other: _____.

DETAILED ACTION

Priority

1. Receipt is acknowledged of papers submitted under 35 U.S.C. 119(a)-(d), which papers have been placed of record in the file.

Claim Objections

2. Claim 15 is objected to because of the following informalities: Claim 15 is not written in proper dependent form. Specifically, claim 15 refers to itself. Appropriate correction is required.

Drawings

3. The drawings are objected to under 37 CFR 1.83(a) because they fail to show specific details enumerated in the description of the drawings as described in the specification. In particular:
 4. Figure 1 fails to indicate "buffer solution 106" as described in paragraph 0026 of the specification.
 5. Figure 2 fails to indicate "size *D*," "z-direction," or the "x-y plane" as described in paragraph 0027 of the specification.
6. Any structural detail that is essential for a proper understanding of the disclosed invention should be shown in the drawing. MPEP § 608.02(d). Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing

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should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Claim Rejections - 35 USC § 112

7. The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

8. Claims 1-8 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claim 1, and by dependency claims 2-8, contain the phrase "and measuring the translocation time of each nucleotide to determine a sequence of the nucleic acid sequence." However, nowhere in the specification is it described how measuring the translocation time of each nucleotide would allow one to determine the nucleic acid

sequence. In fact, the method of claim 1, and by dependency claims 2-8, recites the use of a "rotating electric field" to "control a translocation time of one nucleotide being a multiple of one-fourth of a period of the rotating electric field." Therefore, according to the method, the translocation time per nucleotide is held constant and thus cannot be used to discriminate one nucleotide from another while passing through the pore.

9. Claims 1-15 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

10. Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CAFC 1988). *Wands* states at page 1404,

"Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in *Ex parte Forman*. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

11. The nature of the invention

The claims are drawn to methods to determine the sequence of a nucleic acid by controlling its rate of translocation through a synthetic thin film nanopore, and measuring the fluctuations in the electrical current thus produced. The invention is in a class of invention which the CAFC has characterized as "the unpredictable arts such as chemistry and biology." *Mycogen Plant Sci., Inc. v. Monsanto Co.*, 243 F.3d 1316, 1330 (Fed. Cir. 2001).

12. The breadth of the claims

The claims are broadly drawn to methods for determining the sequence of nucleic acids using a thin film nanopore. These claims would therefore encompass DNA and RNA, single- or double-stranded, including any modifications such as 7-methylguanosine-capped mRNA, methylated DNA, or the various modified residues found in tRNA. The specification does not describe how the claimed methods would allow one to determine the identities of such modifications. With particular regard to double-stranded nucleic acids, the basis described in the specification for discriminating among the 4 bases normally found in nucleic acids (i.e. the unique blockage current signature of each base) would only allow one to determine the sequence of single-stranded nucleic acids. This is because, while the blockage current signature for a GC base pair may be distinguished from that of an AT base pair, one would not be able to deduce which strand of a double-stranded nucleic acid molecule contained G and which contained C, for example.

13. Quantity of experimentation

The quantity of experimentation required by one of ordinary skill in the art to practice the methods claimed by Applicant is large. This is mainly due to the fact that the methods have not been reduced to practice and are based solely on predictions involving computer simulations. Applicant has given no indication of actually fabricating the device required to practice the method. A publication by Applicant subsequent to the filing of the instant application also provides no indication of a working device for practicing the methods claimed (Chen & Peng, 2003). The Court in *In re Ghiron*, 442

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F.2d 985, 991, 169 USPQ 723, 727 (CCPA 1971), made clear that if the practice of a method requires a particular apparatus, the application must provide a sufficient disclosure of the apparatus if the apparatus is not readily available. While Applicant describes the essential features of such an apparatus in the specification, the fabrication of such a device is not described in the specification in such detail as to obviate undue experimentation by one of ordinary skill in the art. The following paragraph discusses some features of the apparatus required to practice the claimed methods that are unpredictable and would therefore require undue experimentation for reduction to practice.

14. The unpredictability of the art and the state of the prior art

The current state of the art indicates that a great deal of further experimentation and inventiveness would be required to implement the methods claimed by Applicant.

For instance, Aksimentiev et al. (2004) states:

"For synthetic nanopores observed blockades presently show irregular shapes (see Fig. 1) due to fluctuations of the reduced current within one blockade event; some blockades have a positive spike of the ionic current above the open pore level at the end of the blockade" (p. 2087, column 1, lines 7-11).

Aksimentiev et al. (2004) cites Applicant's 2003 publication describing the methods of the instant application, and was therefore aware of Applicant's invention, and yet states:

"The manufacturing of actual nanopores and electrical recordings are still at an early stage that needs guidance toward optimal strategies toward the ultimate goal of sequencing DNA" (p. 2095, column 2, lines 29-32).

15. Applicant refers to Li et al. (2001) for a method of forming the nanopore to be used in the method claimed in the instant application. However, the nanopores fabricated by Li et al. (2001) are approximately 10nm in length, a distance which can accommodate 10-15 nucleotides at a time. Therefore, the blockage current measured

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would be the result of the presence of multiple nucleotides in the pore simultaneously and could not be used to identify the sequence of the nucleic acid molecule. This technical challenge (i.e. the length of the pore channel) has still not been completely met, as stated by Heng et al. (2004):

“...the scaling of MOS [metal oxide semiconductor] field effect transistor (MOSFET) designs to nanometer-scale gate length (Sorsch et al., 1998) have produced high-integrity, ultrathin films as thin as 0.7 nm (which is comparable to the spacing between basepairs in DNA) that are suitable for membranes. By combining these two elements, we have already produced ~1-nm diameter pores that are smaller in diameter than DNA in solution, **through membranes ~5-50-nm thick**” (p. 2905, column 2, lines 22-29, emphasis added).

Therefore, even as late as 2004, the technical hurdle of producing a nanopore in a film thin enough to enable single nucleotide resolution based on blockage current has still not been overcome.

16. An additional issue of unpredictability that the specification does not address is the potential for interaction of the nucleic acid with the material of the nanopore.

Aksimentiev et al. (2004) states:

“A strong hydrophobic interaction of the DNA bases with the surface of the pore (Fig. 7) favors an unzipped conformation of a double-stranded DNA inside the pore (Figs. 6 and 9). A significant reduction of the ionic current can be observed even when DNA is not transiting the pore (Fig. 8), such that only part of the ionic current blockade measured experimentally reflects actual DNA translocation events” (p. 2095, column 2, lines 19-26).

The issue of nucleic acid interacting with the pore is also raised by Heng et al. (2004):

“With or without deconvolution, it is evident from the variety of transients observed that the level of the blocking current changes during the time interval of the transient, which we interpret as the molecule interacting with the pore. Molecular dynamics simulations described below and in (Aksimentiev et al., 2004), indicate that the level of the blocking current is correlated to the velocity of the molecule in the pore and with the bulk electrolyte flow accompanying the translocation event. Consequently, we expect that interactions between the molecule, the pore, and the membrane give rise to a nonuniform molecular velocity and bulk electrolyte flow, which are manifested as variations in the blocking current during a single translocation. Moreover, the simulations show that when DNA exits the pore, ions accumulating near the mouth are also released resulting in the positive current spike similar to that observed experimentally on the rising edge of the transient in Fig. 3” (p. 2908, column 1, line 23 through column 2, line 7).

17. Yet another unpredictable feature of the method claimed by Applicant is the effect of the rotating electric field on the perpendicular electric field required both for the translocation of the nucleic acid through the nanopore as well as the electronic signal allowing the identification of each nucleotide passing through the nanopore. At best, the rotating electric field would have no effect on the perpendicular electric field or the electric current passing through the nanopore. At worst, the perpendicular electric field would experience such interference from the rotating electric field that the nucleic acid might not translocate at all, or the electric current passing through the nanopore would be so noisy as to render identification of the translocating nucleic acid sequence impossible. Without actual reduction to practice, it is not possible to predict with reasonable certainty whether the claimed methods would work.

18. Working examples

As Applicant's invention has not been reduced to practice, there are no working examples provided in the specification.

19. Guidance in the specification

The specification provides guidance as to the essential features of the device required for practicing the method, but provides little guidance as to the actual fabrication of such a device. The specification does offer general guidance for optimizing parameters such as the applied voltage, the frequency of the rotating electric field, the use of nucleic acid "tags" to attach to the ends of the nucleic acid molecule to be sequenced, and the modification of certain bases to create more distinction in the blockage current signal.

20. Level of skill in the art

The level of skill in the art is deemed to be high.

21. Conclusion

In the instant application, a method for determining the sequence of nucleic acids is proposed in which one electric field is applied perpendicular to a thin film containing a single synthetic thin film nanopore in order to drive a nucleic acid molecule through the nanopore, while a second rotating electric field controls the rate of translocation of the nucleic acid through the nanopore, thus allowing the change in the electric current through the nanopore to be used as a signature for the identification of each nucleotide as it passes through. While the methods claimed by Applicant might allow for the controlled rate of translocation of the nucleic acid molecule through the nanopore, the likelihood that one of skill in the art could readily practice the methods claimed by Applicant is remote given:

- I. the method has not been reduced to practice
- II. the device required for practice of the methods has not been fabricated
- III. the methods are based on computer simulations that may not adequately reflect all of the unpredictable realities of the system under investigation (i.e. the movements of polynucleotides in solution, the interaction of polynucleotides with the materials of the nanopore, the effects of the rotating electric field on the electronic signals of the nanopore)

IV. the limitations in the current state of the art (chiefly, the lack of a nanopore-containing film thin enough to achieve single nucleotide resolution)

It would therefore require a person of ordinary skill in the art undue experimentation to practice the methods claimed in the instant application. Therefore, rejection of claims 1-15 under 35 U.S.C. 112, first paragraph is proper.

Conclusion

22. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Samuel C. Woolwine whose telephone number is (571) 272-1144. The examiner can normally be reached on 8:30-5:00.

23. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

24. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

scw


JEFFREY FREDMAN
PRIMARY EXAMINER
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